

Cold Hardiness of the Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae)

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ABSTRACT A classical biological control agent, *Harmonia axyridis* (Pallas), is having both beneficial and detrimental impacts in North America. The objective of this study was to evaluate the cold hardiness of *H. axyridis* in North America. Supercooling points and survival at subzero temperatures of field-collected and insectary-reared *H. axyridis* were examined. The mean (\pm SE) supercooling points for eggs and pupae (i.e., nonfeeding stages) were $-27.0 \pm 0.18^\circ\text{C}$ and $-21.3 \pm 0.52^\circ\text{C}$, respectively. The mean supercooling points for larvae and adults (i.e., feeding stages) were -14.17 ± 0.33 and $-11.9 \pm 0.53^\circ\text{C}$, respectively. Sex and color morph (i.e., red: f. *succinea* versus black: f. *spectabilis*) had no effect on the supercooling point of *H. axyridis* adults. Mean supercooling points of *H. axyridis* adults from Minnesota and Georgia were significantly lower during winter months than summer months. The mortality of *H. axyridis* increased significantly after individuals were exposed to temperatures below the mean supercooling point of the population. Supercooling point was a good predictor of cold hardiness. However, the cold hardiness of *H. axyridis* appears to be a poor predictor of its northern distribution.

KEY WORDS *Harmonia axyridis*, cold hardiness, supercooling point, overwintering, low temperature

THE MULTICOLORED ASIAN LADY beetle, *Harmonia axyridis* (Pallas), most likely entered North America through intentional releases for biological control (Gordon 1985). However, Day et al. (1994) argue that *H. axyridis* may have entered through accidental sea-port introductions. The first established population of *H. axyridis* in North America was recorded in 1988 (Chapin and Brou 1991). The establishment of this exotic coccinellid has had both beneficial and detrimental consequences. As a benefit, *H. axyridis* feeds on pest insects of numerous crops, such as pecan (Tedders and Schaefer 1994), apple (Brown and Miller 1998), citrus (Michaud 1999, Michaud 2000, Stuart et al. 2002), and corn (Musser and Shelton 2003). Conversely, evidence suggests that *H. axyridis* may be adversely affecting native natural enemies (Koch 2003) and other nonpest insects, such as the monarch butterfly, *Danaus plexippus* (L.) (Koch et al. 2003). In addition, two unexpected adverse effects of *H. axyridis* are its status as a household nuisance pest (Huelsen et al. 2002), and as an emerging pest in fruit production (Ejlbich 2003, Koch et al. 2004).

The potential geographic extent of impacts by an exotic organism, such as *H. axyridis*, depends on the capacity of the organism to withstand unfavorable environmental conditions, such as temperature ex-

temes (Tauber et al. 1986). Insects in cold regions require some degree of cold hardiness to protect them from low temperatures (Bale 1987, Salt 1961). In general, insects that survive freezing temperatures can be classified as freeze tolerant or freeze intolerant (Salt 1961). Freeze-tolerant insects withstand extracellular ice formation, while freeze-intolerant insects avoid ice formation by supercooling (Baust and Rojas 1985). The supercooling point is defined as the temperature at which body fluids spontaneously freeze when cooled below the melting point (Zachariassen 1985). The supercooling point generally represents the absolute lower lethal temperature for freeze-intolerant individuals, although death may also occur at temperatures above this point as a result of chill injury (Lee 1991, Lee and Denlinger 1985). In addition to the supercooling point, lower lethal temperature and lethal time have been used as indices of cold hardiness (Watanabe 2002).

Adult *H. axyridis* overwinter in mass aggregations in or near prominent objects on the horizon (reviewed by Koch 2003). Watanabe (2002) suggested that *H. axyridis* in Japan might be freeze intolerant with a degree of chill tolerance. Concentrations of a known cryoprotectant, myo-inositol, increased concurrently with a seasonal decrease in the supercooling point of adult *H. axyridis* (Watanabe 2002). However, cold hardiness is affected by several other factors, including geographic location, environmental conditions,

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developmental stage, sex, and age (Sømme 1982, Turnock et al. 1990, Renault et al. 2002). The objective of the current study was to measure the effect of developmental stage, season, color morph (i.e., red: f. *succinea* versus black: f. *spectabilis*), and sex on the supercooling point. Survival at subzero temperatures also was analyzed as an index of cold hardiness of *H. axyridis* in the United States.

Materials and Methods

Supercooling Point Determinations

Supercooling points were measured using surface-contact thermometry, as described by Carrillo et al. (2004). Individuals were attached to a 24-gauge copper-constantan thermocouple using high vacuum grease (Dow Corning, Midland, MI). Insect-thermocouple arrangements were placed inside a solid $19 \times 19 \times 19$ -cm polystyrene cube, and these into a -80°C freezer to cool insects at $\approx 1^\circ\text{C min}^{-1}$ (Carrillo et al. 2004). Supercooling points were determined as the lowest temperature reached before the release of latent heat of fusion. The release of latent heat is observed as an abrupt increase of the body temperature.

Effect of Developmental Stage on Supercooling Point

The relative cold hardiness of *H. axyridis* was examined by determining the supercooling point of each developmental stage. Individuals used in this study were laboratory-reared F_1 progeny of field-collected overwintering adults from St. Paul, Minnesota. Overwintering adults were placed into 60×15 -mm plastic petri dishes and held at 22°C with a photoperiod of 16:8 (L:D) h. Insects were provisioned with an ad libitum supply of drone honey bee diet (Okada and Matsuka 1973) and water. Mating was observed within 5 d of warming. Individual mated females were placed into 60×15 -mm plastic petri dishes and were provisioned with an ad libitum supply of pea aphids, *Acyrtosiphon pisum* (Harris), and water. Offspring of the mated females were individually reared to the desired stage of development in 60×15 -mm plastic petri dishes provisioned with an ad libitum supply of drone honey bee diet and water. Eggs used for supercooling-point measurements were removed from the petri dish using a small camelhair brush. Eggs, first through fourth instars, and pupae were used in the study 24–36 h after molting to the desired stage. Adults were used in the study 72 h after eclosion. Supercooling-point measurements were recorded from 18 eggs, 20 first instars, 17 second instars, 18 third instars, 17 fourth instars, 20 pupae, and 20 adults. Data were not analyzed statistically because each stage of development was measured at separate times because of difficulty in synchronizing the development of all stages tested.

Effect of Sex and Color Morph on Supercooling Point

The effect of sex on the supercooling point of adult *H. axyridis* was examined for field-collected individuals. On 16 April, 15 August, and 9 and 26 September 2002, adult *H. axyridis* were collected near Rosemount, Minnesota. One day after collection, individuals were sexed and their supercooling points were determined. Supercooling points were determined for 10–21 individuals of each sex for each collection date. To determine the effect of color morph on supercooling point, red adults with black spots (f. *succinea*) and black adults with four red spots (f. *spectabilis*) were obtained from The Green Spot (Nottingham, NH) on 29 May 2003. On the following day, the supercooling point was determined for 17 and 16 individuals of the red and black color morphs, respectively. Individuals from The Green Spot were from an insectary-reared colony that was ~ 5 yr old and frequently supplemented with field-collected individuals. Because the black color morphs of *H. axyridis* are rare in North America (LaMana and Miller 1996), particularly in Minnesota (R. L. Koch, unpublished data), we had to rely on the insectary-reared individuals. Data for sex or color morph comparisons were analyzed with analysis of variance (ANOVA) (Proc GLM, SAS Institute 1995). For the sex comparison, sex, collection date, and the interaction between sex and collection date were included in the model. For the color morph comparison, color was the sole predictor in the model.

Effect of Season on Supercooling Point

The effect of season on the supercooling point of adult *H. axyridis* was examined for populations from Minnesota and Georgia. Adult *H. axyridis* used in this study were field collected 24–72 h before measuring the supercooling point. Adults were collected from various locations near St. Paul and Rosemount, Minnesota, and from the United States Department of Agriculture-Agricultural Research Service Fruit and Tree Nut Research Laboratory in Byron, Georgia. Supercooling points of adults from Minnesota were measured on 16 April, 15 August, 26 September, 8 November, 26 November 2002, 7 February, 26 February, 9 April, 26 August, and 15 December 2003. Supercooling points of adults from Georgia were measured on 19 September, 23 November 2002, and 7 February 2003. For each date, supercooling-point measurements were determined from 18 to 42 individuals. The seasonal change of mean supercooling points of individuals from each location was analyzed using ANOVA (Proc GLM, SAS Institute 1995) and the Tukey's Studentized Range test (honestly significant difference [HSD]). Month was the sole predictor used in the ANOVA models. When multiple supercooling-point measurements were taken within a month, the measurements for that month were pooled.

Effect of Subzero Temperatures on Adult Survival

The effect of subzero temperatures on the survival of adult *H. axyridis* was examined for field-collected and insectary-reared individuals. Adults used in this study were either collected near Rosemount, Minnesota, on 26 August 2003 or obtained from The Green Spot on 31 May 2003. Groups of 10 adults were placed into 16 × 150-mm glass test tubes that were closed with a small piece of foam. Test tubes containing adults were placed into the core of a 35 × 35 × 35-cm polystyrene cube with a starting temperature of 27°C. A 24-gauge copper-constantan thermocouple also was placed into each test tube to monitor temperature. The polystyrene cube was then placed into a -80°C freezer to cool the insects at a rate of $\approx 0.3^\circ\text{C min}^{-1}$. Insectary-reared individuals were cooled to 0, -5, -10, -15, -20, -25, or -30°C; and field-collected individuals were cooled to 0, -5, -10, -15, or -20°C. Test tubes were removed from the cube 1 min after reaching the desired temperature and immediately placed into a programmable growth chamber set at 0°C. Ten minutes after the final tube was placed into the growth chamber, insects were warmed to 22°C at a rate of $0.3^\circ\text{C min}^{-1}$. Preliminary data indicated that a 10-min exposure to 0°C was sufficient to equilibrate adults from all temperature treatments to 0°C (data not shown). One test tube was withheld from the cooling treatment and was placed directly into the growth chamber at 22°C as a control. Adults from each treatment and the control were transferred to separate 150 × 15-mm plastic petri dishes provisioned with an ad libitum supply of drone honey bee diet and water. The dishes were held at 22°C and 60–70% RH under a photoperiod of 16:8 (L:D) h. Adult survival was assessed at 24 h after treatment by flipping individuals onto their backs. Individuals were considered functionally dead if they were unable to right themselves within an additional 24 h (i.e., 48 h after treatment). Both trials of this experiment were replicated three times, except for the trial with *H. axyridis* from Minnesota, in which -20°C exposure treatment was replicated twice. The control showed no mortality, so a correction (Abbott 1925) was not necessary. An ANOVA (Proc GLM, SAS Institute 1995) and the Tukey's Studentized Range test (HSD) were used to test for differences in the arcsine square root transformed proportionate mortality for each treatment. The supercooling points of 28 field-collected and 73 insectary-reared individuals were determined for comparison with the results of the survival study. For individuals from each source, the cumulative percentage of individuals supercooling was calculated by summing the number of individuals that supercooled at and above each 1-degree temperature step, and dividing each resulting sum by the total number of individuals measured.

Survival at Various Points on the Supercooling Curve

To examine survival of adult *H. axyridis* at various points along their supercooling curves, we modified the method from Carrillo et al. (2004). Instead of using

high vacuum grease to attach individuals to the thermocouples, a modified 6-ml plastic syringe was used to hold individuals in contact with the thermocouples (Brunnhofer et al. 1991). The cooling rate was achieved, as described above, for the supercooling point studies. Individual body temperatures were monitored using an Omega 2809 digital thermometer (Omega Engineering, Stamford, CT) and graphed using a Fischer Graph Recordall (Fischer Scientific, Springfield, NJ) (Schmid 1988). *H. axyridis* used in this study were laboratory reared under the conditions described above in the study examining the effect of developmental stage on supercooling point. Individuals were cooled to either their supercooling point (i.e., the lowest temperature attained before the release of latent heat of fusion), the peak of the exotherm (i.e., the maximum temperature attained after the release of latent heat of fusion), or the end of the exotherm (i.e., the temperature equal to the supercooling point) (Block et al. 1988). The removal point was randomly determined before each run. Seven individuals were cooled to each of these three points on the supercooling curve. When an individual reached the desired point along the supercooling curve, it was immediately removed from the freezer and placed into 150 × 15-mm plastic petri dishes provisioned with drone honey bee diet and water. The dishes were then placed into a programmable growth chamber at 0°C for 10 min and warmed to 22°C at a rate of $0.3^\circ\text{C min}^{-1}$. After 24 h, survival was assessed as in the study examining the effect of subzero temperatures on adult survival. The percentage of mortality at each removal point was compared with zero based on the overlap of 95% confidence intervals for population proportions with small sample sizes (Kvanli 1988).

Results

Effect of Developmental Stage on Supercooling Point

In rank order, the mean (\pm SE) supercooling point for eggs was $-27.0 \pm 0.18^\circ\text{C}$; pupae, $-21.3 \pm 0.52^\circ\text{C}$; first instars, $-15.4 \pm 0.82^\circ\text{C}$; third instars, $-13.9 \pm 0.53^\circ\text{C}$; fourth instars, $-13.8 \pm 0.60^\circ\text{C}$; second instars, $-13.3 \pm 0.45^\circ\text{C}$; and adults, $-11.9 \pm 0.53^\circ\text{C}$ (Fig. 1).

Effect of Sex and Color Morph on Supercooling Point

The mean supercooling point of adult *H. axyridis* was not significantly affected by sex ($F = 0.51$; $df = 1, 116$; $P = 0.4783$) (Table 1). In addition, the interaction of sex and date was not significant ($F = 0.90$; $df = 3, 116$; $P = 0.4455$). However, the effect of date was significant ($F = 13.18$; $df = 3, 116$; $P < 0.0001$). Color morph did not significantly affect the mean supercooling point of adult *H. axyridis* ($F = 1.13$; $df = 1, 31$; $P = 0.2967$) (Table 1).

Effect of Season on Supercooling Point

The mean supercooling points of *H. axyridis* significantly changed through time for adults from Minne-

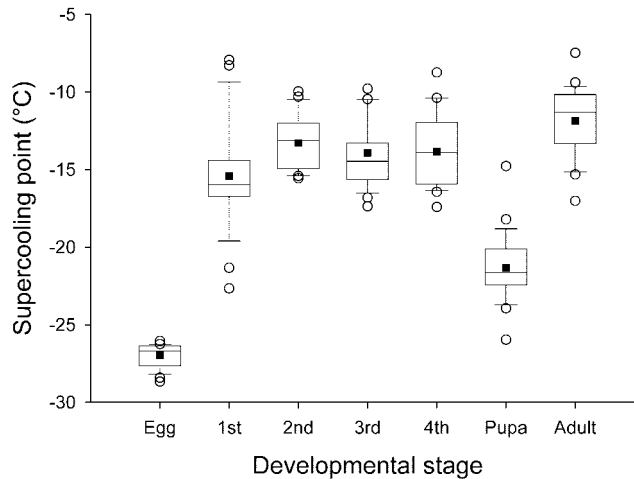


Fig. 1. Effect of developmental stage on the supercooling point of laboratory-reared *H. axyridis*. The center bars of the box plots represent the median; the upper and lower ends of the boxes represent the 25th and 75th percentiles; the whiskers represent the 10th and 90th percentiles; circles represent outliers; and the squares represent the mean.

sota ($F = 73.64$; $df = 7, 257$; $P < 0.0001$) and Georgia ($F = 6.50$; $df = 2, 71$; $P = 0.0026$) (Fig. 2, A and B). For individuals from Minnesota, the mean supercooling point decreased $\approx 13^\circ\text{C}$ from August 2002 to February 2003, and $\approx 11^\circ\text{C}$ from August 2003 to December 2003 (Fig. 2A). For individuals from Georgia, the mean supercooling point decreased $\approx 4^\circ\text{C}$ from November 2002 to February 2003 (Fig. 2B).

Effect of Subzero Temperatures on Adult Mortality

Significant differences in the percentage of mortality were observed among treatments for field-collected individuals from Minnesota ($F = 24.64$; $df = 4, 8$; $P = 0.0001$) and insectary-reared individuals from The Green Spot ($F = 36.93$; $df = 6, 14$; $P < 0.0001$). The insectary-reared *H. axyridis* had a mean supercooling point of $-15.9 \pm 0.40^\circ\text{C}$, with values ranging from -23.5°C to -8.0°C . The field-collected *H. axyridis* had a mean supercooling point of $-8.0 \pm 0.54^\circ\text{C}$, with

values ranging from -18.5°C to -6.7°C . From either source, the percentage of *H. axyridis* that died was not significantly $>0\%$ at temperatures above or near the mean supercooling point (Fig. 3, a and b). After the mean supercooling point was surpassed, a significant increase in mortality ($>50\%$) was observed for individuals from both locations (Fig. 3, a and b). Mortality reached 100% when the minimum value of the supercooling point range was surpassed (Fig. 3, a and b). The curves for the cumulative percentage of individuals supercooling were consistently shifted to the right of the mortality curves (Fig. 3, a and b).

Mortality at Various Points on the Supercooling Curve

The mean supercooling point for individuals tested in this portion of the study was $-11.7 \pm 0.79^\circ\text{C}$. Individuals cooled to their supercooling point showed no mortality (Fig. 4). However, the percentage of mortality was significantly greater than zero ($P <$

Table 1. Effect of sex and color morph on the supercooling point (SCP) of adult *Harmonia axyridis*

Date	Comparison	n	Mean SCP ($^\circ\text{C}$) \pm SE ^c	Range ($^\circ\text{C}$)
16 April 2002	Sex ^a			
	Female	13	-14.2 ± 1.02	$-18.8, -8.2$
15 Aug. 2002	Female	14	-12.4 ± 0.95	$-18.0, -7.6$
	Male	21	-9.8 ± 0.56	$-17.2, -5.2$
9 Sept. 2002	Male	21	-10.1 ± 0.38	$-15.3, -8.2$
	Female	15	-13.6 ± 0.77	$-16.9, -8.2$
26 Sept. 2002	Male	15	-13.8 ± 0.68	$-17.1, -8.3$
	Female	15	-11.3 ± 0.78	$-16.4, -6.6$
29 May 2003	Male	10	-11.1 ± 0.83	$-14.4, -6.2$
	Color ^b			
	Red	17	-16.8 ± 0.53	$-23.5, -10.8$
	Black	16	-17.8 ± 0.73	$-20.1, -10.0$

^a Individuals were field-collected near Rosemount, MN.

^b Individuals were insectary-reared and obtained from The Green Spot, Ltd., Nottingham, NH. The red color morph was *f. succinea*, and the black color morph was *f. spectabilis*.

^c Mean SCP's for sex within dates and color morph did not differ significantly ($P > 0.05$); analysis of variance (Proc GLM, SAS 1995).

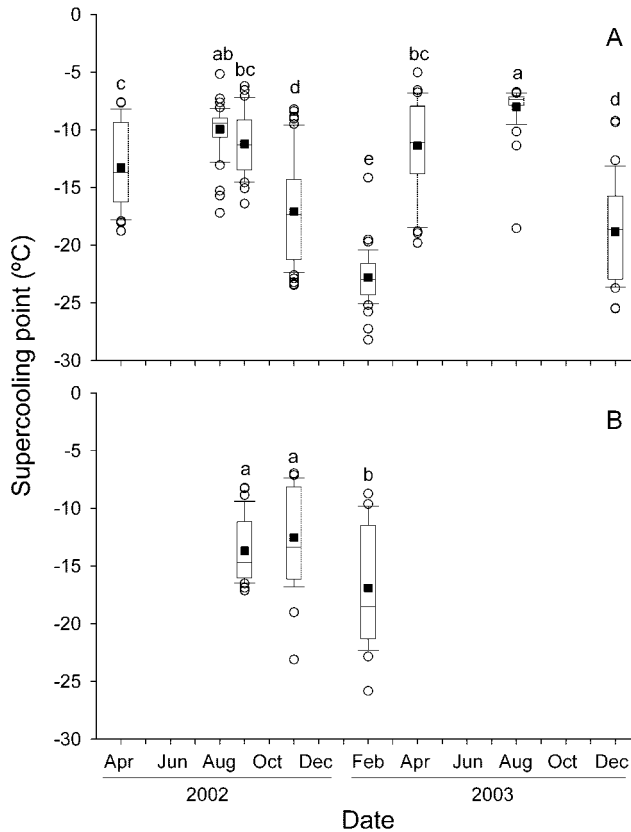


Fig. 2. Effect of season on the supercooling point of field-collected adult *H. axyridis* from (A) Minnesota and (B) Georgia. The center bars of the box plots represent the median; the upper and lower ends of the boxes represent the 25th and 75th percentiles; the whiskers represent the 10th and 90th percentiles; circles represent outliers; and the squares represent the mean. Different lower case letters above box plots indicate significant differences among mean supercooling points (Minnesota, $P < 0.0001$; Georgia, $P = 0.0026$) based on ANOVA and Tukey's Studentized Range test (HSD).

0.05) at the peak (43%) and at the end (57%) of the exotherm (Fig. 4).

Discussion

The ability of *H. axyridis* to overwinter has received attention in the northeastern United States (McClure 1987) and in Japan (Watanabe 2002). Reports of >90% overwintering mortality (McClure 1987) suggest that cold winter temperatures may be an important factor regulating populations of *H. axyridis* from one year to the next. Watanabe (2002) suggested that the ability of *H. axyridis* to survive in more northern latitudes (i.e., colder locations) than Tsukuba, Japan, would be dependent upon the ability of this insect to increase its cold hardiness (e.g., depress the supercooling point) during winter months.

Our results indicate that the supercooling point of *H. axyridis*, as with other freeze-intolerant insects, significantly changes with season (e.g., Fig. 2, a and b). In addition, it appears that developmental stage also may have an effect on the supercooling point of this

coccinellid (e.g., Fig. 1). The mean supercooling points for nonfeeding stages (i.e., eggs and pupae) of *H. axyridis* remained below -20°C , while the mean supercooling points of the feeding stages (i.e., larvae and adults) were warmer than -16°C (Fig. 1). These results suggest that food in the digestive tract may induce ice nucleation, and increase the supercooling point (Salt 1953). Therefore, the lower supercooling points of field-collected adult *H. axyridis* during winter (Fig. 2) (Watanabe 2002) may be partially because of an absence of food in the digestive tract (Iperti and Bértand 2001).

Freeze-intolerant insects die when exposed to temperatures at or below the supercooling point, but some may die at temperatures above the supercooling point, because of chill injury (Lee 1991). For *H. axyridis*, the supercooling point appears to be a good indicator of cold hardiness when mortality is assessed after an exposure period of 1 min. However, Watanabe (2002) found that some prefreeze mortality occurred when adult *H. axyridis* were exposed for a longer period of time (i.e., 24 h). The results of our studies indicate that

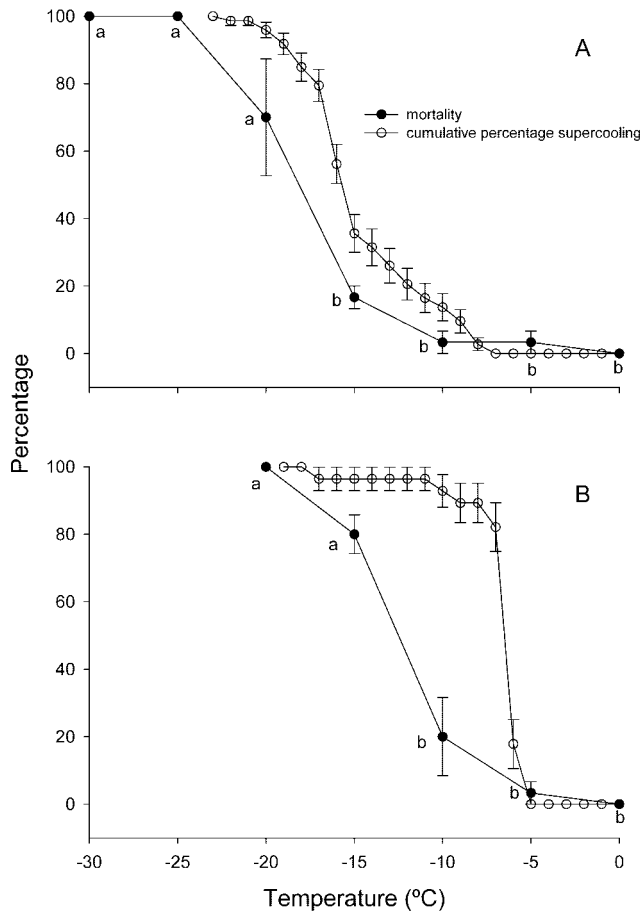


Fig. 3. Mean (\pm SE) percentage of mortality and cumulative percentage of adult *H. axyridis* supercooling at different subzero temperatures for: (A) insectary-reared individuals from The Green Spot; (B) field-collected individuals from Rosemount, Minnesota. For each location, different lower case letters indicate significant differences among means within each mortality curve (field collected, $P = 0.0001$; insectary reared, $P < 0.0001$) based on ANOVA and Tukey's Studentized Range test (HSD).

mortality of *H. axyridis* did not occur immediately at the supercooling point, but increased with time after the supercooling point was reached. Similar results have been observed for other freeze-intolerant insects in which mortality was proportional to the amount of ice formed inside the body (Salt 1953, Block et al. 1988). In our study examining the effect of subzero temperatures on mortality of *H. axyridis* adults, the difference in the shapes of the curves for cumulative percentage of individuals supercooling from Minnesota compared with Georgia (Fig. 3) may be because of the difference in the number of individuals used to characterize the distribution (73 individuals from Minnesota versus 28 individuals from Georgia). The ability of *H. axyridis* to survive after exposure to their supercooling point may be an artifact of minimal exposure times used in laboratory studies, as recognized by Salt (1953) while working with other freeze-intolerant insects. In other words, survival of individuals after a short-duration exposure to the supercooling

point in laboratory studies does not necessarily indicate freeze tolerance, and further investigation (e.g., increased exposure time) may be required to categorize cold hardiness.

The presence of *H. axyridis* has been confirmed in parts of the northern United States (Koch and Hutchison 2003) and southern Canada (Coderre et al. 1995, McCorquodale 1998). In these locations, minimum air temperatures (Kaliyan et al. 2003) can exceed the minimum supercooling points observed in this study and should be lethal to *H. axyridis*. Thus, local air temperature alone appears to be a poor predictor of the distribution of *H. axyridis*. *H. axyridis* adults most likely find microclimates for overwintering that provide protection from extreme low temperatures. *H. axyridis* generally shows hypsotactic orientation (i.e., movement toward prominent objects) during its fall movement to overwintering sites (Obata 1986). At the overwintering sites, mass aggregations of *H. axyridis* adults are formed (Tanigishi 1976) in dark,

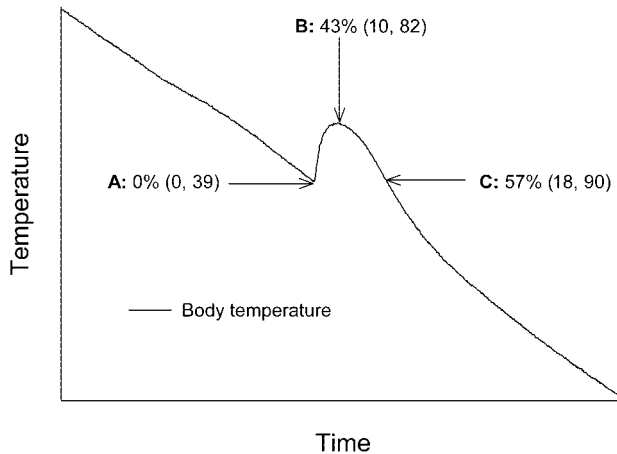


Fig. 4. Percentage of mortality (95% confidence interval) of adult *H. axyridis* at various points on a typical supercooling curve: (A) supercooling point (i.e., the lowest temperature point attained before the release of latent heat of fusion); (B) peak of exotherm (i.e., the maximum temperature attained after the release of latent heat of fusion); and (C) end of exotherm (i.e., the temperature equal to the supercooling point). Arrows indicate points at which individuals were removed from cooling. Percentage of mortality of individuals removed at points (B) and (C) differed significantly from zero ($P < 0.05$) based on the overlap of 95% confidence intervals for population proportions with small sample sizes.

concealed locations (Sakurai et al. 1993). *H. axyridis* adults also overwinter in leaf litter (Obata 1986; R. L. Koch, unpublished data), where temperatures may be less extreme (Leather et al. 1993). Therefore, the capacity of *H. axyridis* to survive winter conditions in northern locations may be more related to the availability of quality overwintering sites than to its capacity to increase cold hardiness.

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References Cited

- Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* 18: 265–267.
- Bale, J. S. 1987. Insect cold hardiness: freezing and supercooling—an ecophysiological perspective. *J. Insect Physiol.* 33: 899–908.
- Baust, J. G., and R. R. Rojas. 1985. Insect cold hardiness: facts and fancy. *J. Insect Physiol.* 31: 755–759.
- Block, W., Y. Z. Erzincliglu, and M. R. Worland. 1988. Survival of freezing in *Calliphora* larvae. *CryoLetters* 9: 86–93.
- Brown, M. W., and S. S. Miller. 1998. Coccinellidae (Coleoptera) in apple orchards of eastern West Virginia and the impact of invasion by *Harmonia axyridis*. *Entomol. News* 109: 136–142.
- Brunnhöfer, V., O. Nedvěd, and M. Hodková. 1991. Methodical improvement for measuring of supercooling point in insects. *Acta Entomol. Bohemoslov.* 88: 349–350.
- Carrillo, M. A., N. Kaliyan, C. A. Cannon, R. V. Morey, and W. F. Wilcke. 2004. A simple method to adjust cooling rates for supercooling point determination. *CryoLetters* 25: 155–160.
- Chapin, J. B., and V. A. Brou. 1991. *Harmonia axyridis* (Pallas), the third species of the genus to be found in the United States (Coleoptera: Coccinellidae). *Proc. Entomol. Soc. Wash.* 93: 630–635.
- Coderre, D., É. Lucas, and I. Gagné. 1995. The occurrence of *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae) in Canada. *Can. Entomol.* 127: 609–611.
- Day, W. H., D. R. Prokrym, D. R. Ellis, and R. J. Chianese. 1994. The known distribution of the predator *Propylea quatuordecimpunctata* (Coleoptera: Coccinellidae) in the United States, and thoughts on the origin of this species and five other exotic lady beetles in eastern North America. *Entomol. News* 105: 224–256.
- Ejbich, K. 2003. Producers in Ontario and northern U.S. bugged by bad odors in wine. *Wine Spectator*, 15 May: 16.
- Gordon, R. D. 1985. The Coleoptera (Coccinellidae) of America north of Mexico. *J. New York Entomol. Soc.* 93: 1–912.
- Huelsman, M. F., J. Kovach, J. Jasinski, C. Young, and B. Easley. 2002. Multicolored Asian lady beetle (*Harmonia axyridis*) as a nuisance pest in households in Ohio, pp. 243–250. *In Proceedings of the 4th International Conference on Urban Pests*, 7–10 July 2002, Charleston, SC. Pocahontas Press, Blacksburg, VA.
- Iperti, G., and E. Bértand. 2001. Hibernation of *Harmonia axyridis* (Coleoptera: Coccinellidae) in south-eastern France. *Acta Soc. Zool. Bohem.* 65: 207–210.
- Kaliyan, N., W. F. Wilcke, R. V. Morey, M. A. Carrillo, and C. A. Cannon. 2003. Using low temperatures to control Indian meal moth, *Plodia interpunctella* (Hübner), in stored corn. Paper No. 036154. American Society of Agricultural Engineers (ASAE) Annual Meeting, Las Vegas, NV, 27–30 July 2003. ASAE, St. Joseph, MO. <http://asae.frymulti.com/request2.asp?JID=5&AID=15416&CID=Inv2003&T2>.
- Koch, R. L. 2003. The multicolored Asian lady beetle, *Harmonia axyridis*: a review of its biology, uses in biological control, and non-target impacts. *J. Insect Sci.* 3: 1–16.

- Koch, R. L., E. C. Burkness, S. J. Wold Burkness, and W. D. Hutchison. 2004. Phytophagous preferences of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) to autumn ripening fruit. *J. Econ. Entomol.* 97: 539–544.
- Koch, R. L., and W. D. Hutchison. 2003. Phenology and blacklight trapping of the multicolored Asian lady beetle (Coleoptera: Coccinellidae) in a Minnesota agricultural landscape. *J. Entomol. Sci.* 38: 477–480.
- Koch, R. L., W. D. Hutchison, R. C. Venette, and G. E. Heimpel. 2003. Susceptibility of immature monarch butterfly, *Danaus plexippus* (Lepidoptera: Nymphalidae: Danainae), to predation by *Harmonia axyridis* (Coleoptera: Coccinellidae). *Biol. Control* 28: 265–270.
- Kvanli, A. H. 1988. *Statistics: a computer integrated approach*. West Publishing, St. Paul, MN.
- LaMana, M. L., and J. C. Miller. 1996. Field observations on *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae) in Oregon. *Biol. Control* 6: 232–237.
- Leather, S. R., K.F.A. Walters, and J. S. Bale. 1993. *The ecology of insect overwintering*. Cambridge University Press, Cambridge, MA.
- Lee, R. E., Jr. 1991. Principles of insect low temperature tolerance, pp. 17–46. *In* R. E. Lee, Jr., and D. L. Denlinger (eds.), *Insects at low temperature*. Chapman & Hall, New York.
- Lee, R. E., Jr., and D. L. Denlinger. 1985. Cold tolerance in diapausing and non-diapausing stages of the flesh fly, *Sarcophaga crassipalpis*. *Physiol. Entomol.* 10: 309–315.
- McClure, M. S. 1987. Potential of the Asian predator, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), to control *Matsucoccus resinosa* Bean and Godwin (Homoptera: Margarodidae) in the United States. *Environ. Entomol.* 16: 224–230.
- McCorquodale, D. B. 1998. Adventive lady beetles (Coleoptera: Coccinellidae) in eastern Nova Scotia, Canada. *Entomol. News* 109: 15–20.
- Michaud, J. P. 1999. Sources of mortality in colonies of brown citrus aphid, *Toxoptera citricida*. *BioControl* 44: 347–367.
- Michaud, J. P. 2000. Development and reproduction of ladybeetles (Coleoptera: Coccinellidae) on the citrus aphids *Aphis spiraeicola* Patch and *Toxoptera citricida* (Kirkaldy) (Homoptera: Aphidae). *Biol. Control* 18: 287–297.
- Musser, F. R., and A. M. Shelton. 2003. Predation of *Ostrinia nubilalis* (Lepidoptera: Crambidae) eggs in sweet corn by generalist predators and the impact of alternative foods. *Environ. Entomol.* 32: 1131–1138.
- Obata, S. 1986. Determination of hibernation site in the ladybird beetle, *Harmonia axyridis* Pallas (Coleoptera, Coccinellidae). *Kontyu* 54: 218–223.
- Okada, I., and M. Matsuka. 1973. Artificial rearing of *Harmonia axyridis* on pulverized drone honey bee brood. *Environ. Entomol.* 2: 301–302.
- Renault, D., C. Salin, G. Vannier, and P. Vernon. 2002. Survival at low temperatures in insects: what is the ecological significance of the supercooling point? *CryoLetters* 23: 217–228.
- Sakurai, H., Y. Kumada, and S. Takeda. 1993. Seasonal prevalence and hibernating-diapause behavior in the lady beetle, *Harmonia axyridis*. *Res. Bull. Fac. Agric., Gifu Univ.* 58: 51–55.
- Salt, R. W. 1953. The influence of food on cold hardiness of insects. *Can. Entomol.* 85: 261–269.
- Salt, R. W. 1961. Principles of insect cold-hardiness. *Annu. Rev. Entomol.* 6: 55–74.
- SAS Institute. 1995. *SAS/STAT user's guide, version 6, 4th ed.* SAS Institute, Cary, NC.
- Schmid, W. D. 1988. Supercooling and freezing in winter dormant animals. *In* R. W. Peifer (ed.), *Tested studies for laboratory teaching. Proceedings, Ninth Workshop/Conference of the Association for Biology Laboratory Education (ABLE St. Paul, MN, 15–19 June 1987)*. Kendall/Hunt, Dubuque, IA.
- Sømme, L. 1982. Supercooling and winter survival in terrestrial arthropods. *Comp. Biochem. Physiol.* 73A: 519–543.
- Stuart, R. J., J. P. Michaud, L. Olsen, and C. W. McCoy. 2002. Lady beetles as potential predators of the root weevil *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Florida citrus. *Fla. Entomol.* 85: 409–416.
- Tanigishi, K. 1976. Hibernation of the lady beetle, *Harmonia axyridis*. *Insectarium* 13: 294–298.
- Tauber, M. J., C. A. Tauber, and S. Masaki. 1986. *Seasonal adaptations of insects*. Oxford University Press, New York.
- Tedders, W. L., and P. W. Schaefer. 1994. Release and establishment of *Harmonia axyridis* (Coleoptera: Coccinellidae) in the southeastern United States. *Entomol. News* 105: 228–243.
- Turnock, W. J., P. M. Reader, and G. K. Bracken. 1990. A comparison of the cold hardiness of populations of *Delia radicum* (L.) (Diptera: Anthomyiidae) from southern England and the Canadian Prairies. *Can. J. Zool.* 68: 830–835.
- Watanabe, M. 2002. Cold tolerance and *myo*-inositol accumulation in overwintering adults of a lady beetle, *Harmonia axyridis* (Coleoptera: Coccinellidae). *Eur. J. Entomol.* 99: 5–9.
- Zachariassen, K. E. 1985. Physiology of cold tolerance in insects. *Physiol. Rev.* 65: 799–832.

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